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ORIGINAL ARTICLE

Altitudinal Variation in Digestive Tract Lengh in *Feirana quadranus*

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Abstract Selective pressures favor variation in organ size in response to environmental changes and evolutionary process. In particular, changes in environmental temperature and rainfall at different altitudes often affect food resources, thereby mediating energy intake and allocation. The digestive tract provided a functional relationship between energy intake and allocation, of which gut morphology was associated with diet changes and food quality under different environments. Here we studied altitudinal variation in the digestive tract across four *Feirana quadranus* populations and tested the hypothesis that relative size of digestive tract should increase with increasing altitude. The results showed that although significant variation in length of the digestive tract was observed in females among populations, altitudinal variation in relative length of digestive tract or gut was non-significant. In addition, the digestive tract length was not correlated with temperature and precipitation across the four populations. Our findings suggest that individuals living in low-temperature and -precipitation populations at high altitudes did not display longer digestive tract than high temperature and precipitation populations at low altitudes, possibly because of small populations or sample sizes.

Keywords altitude, *Feirana quadranus*, digestive tract, physiology

1. Introduction

Environmental changes producing selective pressures can lead to variation in morphology, physiology and behavior in organisms (Liao and Lu, 2010; Liao *et al*., 2013a; Wu *et al*., 2016; Tanner *et al*., 2017; Shultz and Burns, 2017; DeMelo and Masunari, 2017; Alton *et al*., 2017; Signor *et al*. 2017; Liao *et al*., 2018; Wang and Liao, 2018; Qin *et al*., 2018; Cai *et al*., 2018; Liu *et al*., 2018; Joseph *et al*., 2018; DeCasien *et al*., 2018). In particular, phenotypically plastic responses to increased altitude and/or latitude exist in several organs (e.g., digestive tract, muscles, brain, liver, heart and lungs; Piersma *et al*., 1999; Naya *et al*., 2009; Jin *et al*., 2015; Jin *et al*., 2016; Zhong *et al*., 2017; Gu *et al*., 2017; Yang *et al*., 2017; Zhao *et al*., 2018; Mai and Liao, 2019). For instance, environmental temperature and rainfall changes often affect food resources, thereby mediating energy intake and allocation (Luo *et al*., 2017). As a result, relatively lower temperature and less rainfall at higher altitudes and/or latitudes are often associated with a relatively longer digestive tract, and larger liver, heart and lungs (Naya *et al*., 2009; Lou *et al*. 2013; Ma *et al*., 2016; Zhong *et al*., 2017).

A functional relationship between energy intake and allocation is provided by the digestive tract where gut plasticity and digestive performance may affect growth,

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reproduction and survival of individuals (McWilliams and Karasov, 2001; Liao *et al*., 2016). Under different environmental conditions, variation in gut morphology in individuals responds environmental stress to improve the local adaptability because diet changes and food quality are associated with gut dimensions (Penry and Jumars, 1987; Naya and Bozinovic, 2004; Naya *et al*., 2009; Li *et al*., 2016; Wei *et al*., 2017). For instance, the consumption of food with high contents of indigestible materials in frogs leads to an increase in gut length (Naya *et al*., 2009; Lou *et al*., 2013).

Like birds and mammals, amphibians can mediate gut length across different environments. Variation in gut morphology in frogs at an inter-populational level in association with temperature and precipitation has been reported recently (Naya *et al*., 2009; Lou *et al*., 2013; Ma *et al*., 2016). For instance, Naya *et al*. (2009) found that digestive tract length is negatively correlated with altitude in the Andean toad (*Bufo spinulosus*) while Lou *et al*. (2013) found a positive correlation between digestive tract and altitude in the Yunnan frog (*Pelophylax pleuraden*). In addition, an increase in digestive tract length is linked to decreased temperature and precipitation in *B. spinulosus* (Naya *et al*., 2009). However, the length of the digestive tract increases with temperature and decreases with precipitation in the Andrew's toad (*B. andrewsi*) among ten populations (Ma *et al*., 2016). Hence, more studies estimating the digestive tract variation across environmental gradients in frogs are needed.

The swelled vent frog (*Feirana quadranus*) inhabits montane streams throughout the Qinling–Daba Mountains, and whose habitats are located from 335-1830 m a.s.l. (Fei *et al*., 2010; Zhong *et al*., 2018). Egg-laying extends from early April to mid-May, and this species is classified as a prolonged breeder (Wells, 1977). In this species, the relative testis size does not increase with altitude among four populations (Tang *et al*., 2018). Moreover, the Qinling-Daba Mountains hosted three refugia for these frogs during the last glacial maximum (Wang *et al*., 2012). So far, little is known about the association of the digestive tract with altitude. In the present study, we investigated altitudinal variation in the digestive tract across four *F. quadranus* populations. Because the lower temperature and less rainfall at higher altitudes can limit food availability and energy intake (Naya *et al*., 2009), larger digestive tract at higher altitudes would be predicted. Here, we tested the hypothesis that the relative size of the digestive tract in *F. quadranus* should increase with increased altitude among four populations.

2. Materials and Methods

A total of 91 individuals (50 males and 41 females) were collected from four populations located in the Qinling Mountains in western China during the breeding season along an 839 m altitudinal gradient from April to June 2017 (Figure 1; Table 1). All individuals were captured in streams by hand at night with a 12-V flashlight and then brought to the laboratory. We observed the breeding state of all individuals and allowed them to complete mating prior to examining. We kept them at room temperature in an individual rectangular tank ($1 \times 0.5 \times 0.8$ m^3, L × W × H) with freshwater of 2 cm depth (Yang *et al*., 2018). We did not give them food prior to euthanizing. All individuals were euthanized by single-pithing (Mai *et al*., 2017a, b; Wu and Liao, 2017). All experiments involving the sacrifice of these live animals were approved by the Animal Ethics Committee at China West Normal University and also specimens were stored in museums

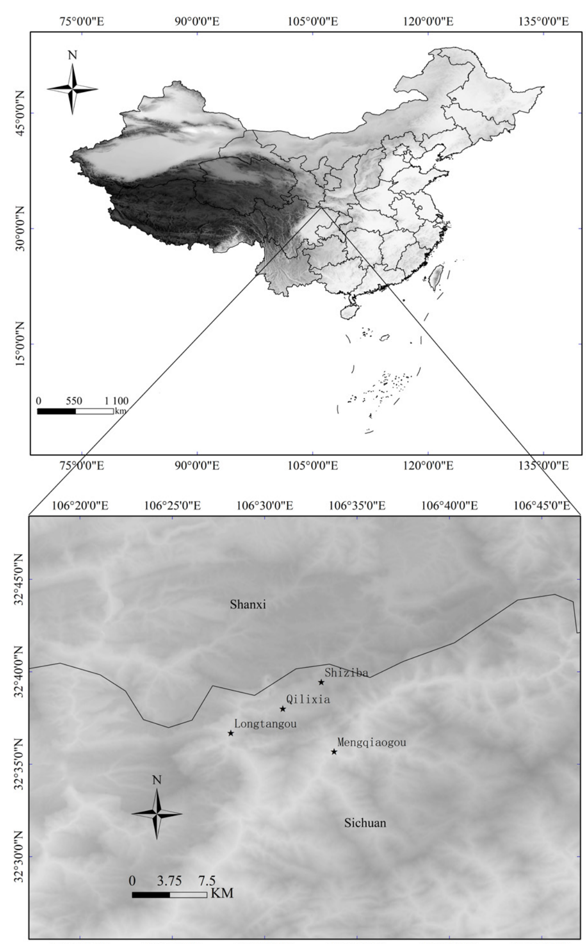

Figure 1 The depicting map of study sites among four *Feirana quadranus* populations in Micangshan Nature Reserve of Sichuan in western China.

Table 1 Altitude, temperature, precipitation, difference in mean body size, digestive tract length between males and females within each *Feirana quadranus* population. *P* indicates significant difference.

Variables	Temperature (°C)	Precipitation (mm)	Altitude (m)	Body size (mm)				Digestive tract (mm)			
				Males	Females	*Z*	*P*	Males	Females	*Z*	*P*
Mengqiaogou	14.7	935	804	76.0 ± 6.9 n = 15	84.8 ± 8.4 n = 11	−2.787	0.004	137.7 ± 36.7 n = 15	166.5 ± 34.7 n = 11	−2.112	0.035
Longtangou	13.1	951	977	54.4 ± 7.4 n = 6	61.3 ± 11.7 n = 7	−1	0.317	94.3 ± 20.5 n = 6	113.6 ± 17.0 n = 7	−1.857	0.063
Qilixia	11.4	976	1289	75.8 ± 11.8 n = 16	80.2 ± 10.2 n = 19	−0.629	0.529	117.6 ± 18.9 n = 16	150.1 ± 30.5 n = 19	−2.715	0.007
Shiziba	9.4	1007	1674	71.7 ± 8.6 n = 9	76.6 ± 19.2 n = 5	−0.867	0.386	95.6 ± 15.6 n = 9	124.4 ± 42.8 n = 5	−1	0.317

of China West Normal University. Body size (snout-vent length: SVL) was measured to the nearest 0.1 mm using a vernier caliper (Liao *et al*., 2015). We dissected out the gonads to determine the sex (Tang *et al*., 2018), then stored them in 4% neutral buffered formalin (Jin *et al*., 2015; Gu *et al*., 2017).

After four weeks, we dissected out the digestive tract of each specimen and measured their length (i.e., the beginning of the esophagus to vent length) to 0.01 mm of precision with a vernier caliper. The entire digestive tract was aligned along a caliper without stretching it, and the digestive tract length was recorded (Lou *et al*., 2013; Liao *et al*., 2016). All dissections and measurements were performed by Huang Jin. We also collected data on average annual temperature and precipitation from http://www.worldclim.org/ (Table 1).

Body size and digestive tract were $\log_{10}$-transformed to improve homogeneity of variances. We first tested differences in digestive tract length between males and females using Mann-Whiney *U*-test. To test for differences in body size and digestive tract for both sexes among populations, we treated body size and digestive tract as dependent variable, and altitude as a fixed factor using Kruskal-Wallis *H*-test. To test for altitudinal variation of relative digestive tract, we used linear general models (GLMs) treating digestive tract as a dependent variable, altitude as fixed a factor, body size and sex as covariates. We also treated digestive tract as a dependent variable, and temperature, precipitation, sex and body size as independent variables to analyze the effects of temperature and precipitation on digestive tract length among populations using a multiple regression analysis.

3. Results

Body size, digestive tract significantly differed between males and females for the Mengqiaogou population (Table 1). For both sexes, there was a significant difference in body size among populations (Kruskal-Wallis *H*-test; males: $\chi^2 = 11.200$, $df = 3$, $P = 0.011$; females: $\chi^2 = 16.392$, $df = 3$, $P = 0.001$). The digestive tract length also displayed significant differences in males ($\chi^2 = 9.580$, $df = 3$, $P = 0.022$) and females across four populations ($\chi^2 = 16.279$, $df = 3$, $P = 0.001$).

The GLMs revealed that variation in relative length of digestive tract was affected by altitude ($F_{3,91} = 5.205$, $P = 0.002$), but not increase with it. The relative length of digestive tract was affected by the sex ($F_{1,91} = 12.438$, $P = 0.001$) and body size (Figure 2; $F_{1,91} = 14.694$, $P < 0.001$). Multiple regression analysis revealed that digestive tract length did not correlate with precipitation ($t = 1.246$, $P = 0.216$). However, the digestive tract length was positively correlated with environmental temperature ($t = 3.595$, $P = 0.001$), sex ($t = 3.471$, $P < 0.001$) and body size ($t = 5.817$, $P < 0.001$).

4. Discussion

Our study demonstrates significant variation in body size and digestive tract length in both females and males among four populations. We found non-significant differences in body size, digestive tract length between males and females for all populations except for the Mengqiaogou population. The length of digestive tract is positively correlated with body size. However, inconsistent with the prediction of digestion theory, the relative digestive tract length does not increase with the altitude of the corresponding study sites. In addition, relative digestive tract length is not correlated with precipitation.

Food quality difference affects the length of the digestive tract between males and females in Grouse and Ptarmigan (Moss, 1983). Previous studies have suggested that the digestive tract significantly differs between males and females in anurans. For instance, females have relatively longer digestive tracts than males in *P.*

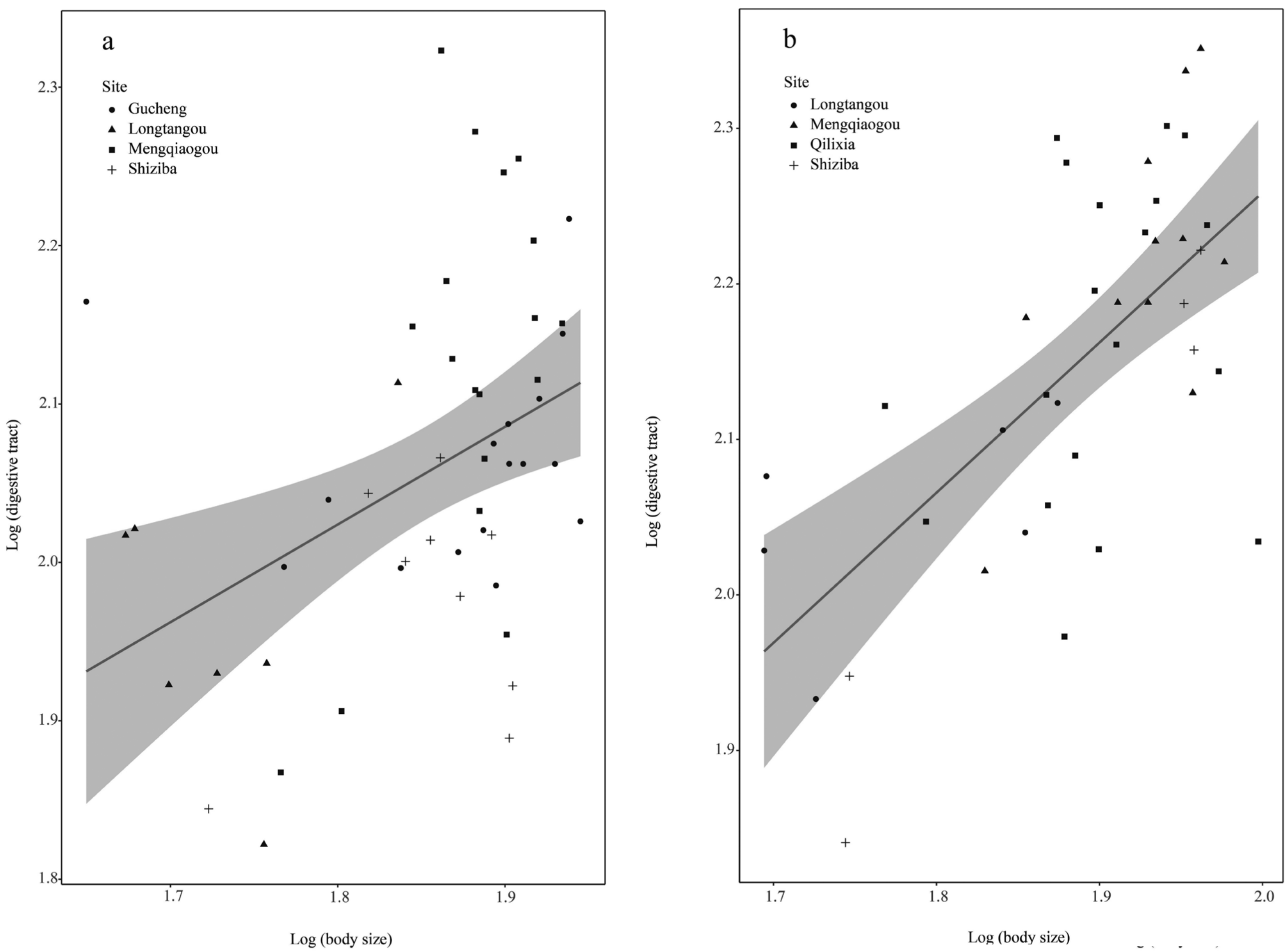

Figure 2 The relationship between length of digestive tract (mm) and body size (mm) among four *Feirana quadranus* populations in Micangshan Nature Reserve of Sichuan in western China (a: male; b: female).

pleuraden (Lou *et al*., 2013) and *Fejervarya limnocharis* (Wang *et al*., 2017). Different energy requirements may result in the differences in digestive tract per unit body mass between the sexes (Pulliainen, 1976). Hence, longer digestive tracts in female frogs may also be explained by different energy requirements, because females require more energy for offspring production. Although we only found that females had longer digestive tract than males in Mengqiaogou population, females displayed relatively larger digestive tract than males using GLMs and multiple regression analysis, suggesting that more energy requirements in female can promote evolution of longer digestive tract in *F. quadranus*.

Animals can adjust their morphology and organ size in responses to environmental changes (Hammond *et al*., 1999; Liu *et al*., 2011; Liao *et al*. 2011; Lou *et al*., 2012; Liao 2013; Liao *et al*. 2013b; Zeng *et al*., 2014; Jiang *et al*., 2015; Mai *et al*., 2017a,b; Zeng *et al*. 2016; Lüpold *et al*., 2017; Pascoal *et al*., 2017; Yang *et al*., 2018; Inostroza-Michael *et al*., 2018; Zhang *et al*., 2018; Zhong *et al*., 2018; Møller *et al*., 2018). In particular, animals mediate their digestive tracts to deal with the changed environments (Hammond *et al*., 1999). There is evidence that individuals from populations that consume greater plant materials exhibit longer guts than those from populations that mainly predate on seeds in the bank voles (*Clethrionomys glareolus*) (Hansson, 1985). Meanwhile, environmental variations directly affect food availability in anurans (reviewed in Morrison and Hero, 2003; Luo *et al*., 2017). There is evidence supporting that, for three species of anurans (*B. spinulosus*, *P. pleuraden*, *F. limnocharis*), decreased temperature may result in decreasing animal-based foods and increasing plant-based foods (Naya *et al*., 2009; Lou *et al*., 2013; Wang *et al*., 2017). Consequently, individuals living higher temperature at lower altitudes should possess shorter guts than individuals living lower temperature at higher altitudes for the three species. However, although

we found significant differences in digestive tract in females and males among *F. quadranus* populations, the individuals from highest altitude did not possess the longest digestive tract.

Previous studies have suggested the altitudinal increase in length of the digestive tract in some anurans species (Naya *et al*., 2009; Lou *et al*., 2013; Wang *et al*., 2017). An increasing in indigestible materials and/or more diverse diets may also drive the increased relative size of the digestive tract in high altitudes for these species. For *F. limnocharis*, individuals foraging on diets that are less diverse nutritious likely experiencing lower temperatures and shorter annual activity periods in high altitudes exhibit an increase of length of the digestive tract (Wang *et al*., 2017). In this study, the relative size of the digestive tract did not display increases with increased altitudes. The non-significant difference in the digestive tract length may be related to the non-significant diversity of nutritious of the contents of stomach among populations. Future study need address variations in prey species of three main arthropod families within each population. In addition, we did not find correlations between digestive tract and precipitation.

Gut size variations among *B. spinulosus* populations suggest that variations in abiotic environments (e.g., temperature, water availability, soil quality) may make biotic conditions to produce changes (e.g., vegetation cover, prey availability), thereby mediating individuals' gut morphology (Naya *et al*., 2009). However, our conclusions need a note of caution because the populations and the sample sizes from the studied populations were low for either of the sexes. Future studies need more populations and sample sizes to gain insight into causes of the digestive tract length variation in this species.

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